11

AAMRL-TR-85-070

ACUTE TOXICITY STUDIES ON TWO AIR FORCE HYDRAULIC FLUIDS (MLO 82-233 AND MLO 82-585)

E. R. Kinkead
J. R. Horton
C. L. Gaworski

UNIVERSITY OF CALIFORNIA, IRVINE
P.O. BOX 31009, OVERLOOK BRANCH
DAYTON, OHIO 45431-0009

R. A. Solomon, MAJ, USAF, BSC

HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOVEMBER 1985

Approved for public release; distribution unlimited.

HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

TECHNICAL REVIEW AND APPROVAL

AAMRL-TR-85-070

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Bruce O. Stuart

BRUCE O. STUART, PhD
Director Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

AD-A163174

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) AAMRL-TR-85-070	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION University of California 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION AAMRL, Toxic Hazards Division
6c. ADDRESS (City, State and ZIP Code) Overlook Branch, P.O. Box 31009 Dayton, OH 45431	7b. ADDRESS (City, State and ZIP Code) AMD, AFSC Wright-Patterson AFB, OH 45433
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER F33615-80-C-0512
8c. ADDRESS (City, State and ZIP Code)	10. SOURCE OF FUNDING NOS. PROGRAM ELEMENT NO.: 622027 · PROJECT NO.: 6302 · TASK NO.: 01 · WORK UNIT NO.: 04
11. TITLE (Include Security Classification) Acute Toxicity Studies of Two Air Force	

12. PERSONAL AUTHOR(S)
E. R. Kinkead, J. R. Horton, C. L. Gaworski, and R. A. Salomon

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
Technical Report	FROM 4/83 TO 3/84	November 1985	23

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)			
FIELD	GROUP	SUB. GR.	Acute Toxicity	Irritation	Chickens	Rats
			Inhalation	Oral	Guinea Pigs	
			Dermal	Acute Neurotoxicity	Rabbits	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This report describes results of investigations into the acute toxic properties of two operational Air Force hydraulic fluids, MLO 82-233 and MLO 82-585. Various routes of administration were investigated including inhalation, dermal, and oral to rats, guinea pigs, rabbits, and chickens.

No acute toxicity occurred in rats with either hydraulic fluid as a result of oral or inhalation exposure at the maximum concentration tested. Dermal exposure of rabbits resulted in no mortalities with either hydraulic fluid. One hydraulic fluid, MLO 82-585, was found to be a moderate skin irritant but neither fluid caused ocular irritation nor did they produce a sensitization response in guinea pigs. Neither fluid would be considered to be a delayed neurotoxin.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☒ DTIC USERS ☐	21. ABSTRACT SECURITY CLASSIFICATION

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
M. K. Pinkerton	(513) 255-5364	AAMRL/THT

DD FORM 1473, 83 APR   EDITION OF 1 JAN 73 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory program being conducted in the Toxic Hazards Research Unit (THRU). This document constitutes a final technical report on the hydraulic fluids, MLO 82-233 and MLO 82-585. The research covered in this report began in April 1983 and was completed in March 1984 and was performed in part under Air Force Contract Number F33615-80-C-0512. M. K. Pinkerton served as contract technical monitor for the Air Force Aerospace Medical Research Laboratory.

J. D. MacEwen, Ph.D. served as Laboratory Director for the THRU of the University of California, Irvine and as co-principal investigator with T. T. Crocker, M.D., Professor, Department of Community and Environmental Medicine. Acknowledgement is made to R. S. Bowers, R. K. Blasingame, and J. L. Monroe for their significant contributions and assistance in the preparation of this report.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACUTE TOXICITY STUDIES ON TWO AIR FORCE HYDRAULIC FLUID (MLO 82-233 AND MLO 82-585)

INTRODUCTION

The Toxic Hazards Research Unit was requested to evaluate the acute toxicity of two hydraulic fluids, MLO 82-233 and MLO 82-585. The two hydraulic fluids, currently in use, meet the Air Force specifications MIL-H-83282 and MIL-H-5606, respectively.

Because of the possibility of exposure of military and civilian personnel to these triarylphosphate containing mixtures during manufacturing, processing, and transportation, the Air Force requested definition of the toxic hazards of the two hydraulic fluids. MIL-H-5606 specifies a petroleum base hydraulic fluid intended for use in aircraft, missile, and ordinance, while MIL-H-83282 defines a fire-resistant, synthetic hydrocarbon base hydraulic fluid for aircraft use. Both hydraulic fluids contain varying amounts of tricresylphosphate (TCP), the amount of which is not limited in the specifications although the ratio of the ortho isomer (TOCP) is limited to 1% of the total TCP used.

Many organophosphorus compounds have been found to cause delayed neurotoxic effects in man (Doull et al., 1979). A single exposure to a neurotoxic organophosphorus compound has been reported capable of producing axonal damage after a delay of 8-10 days. Low level nerve injury may occur in humans after chronic exposure to these compounds. Similar neurotoxic effects have been demonstrated in adult chickens and cats after exposure to triorthocresylphosphate (TOCP) by Beresford and Glees (1963).

Tests conducted with MLO 82-233 and MLO 82-585 included acute oral, dermal, and inhalation toxicity as well as eye and skin irritation and sensitization. Since both fluids contain TCP, an acute neurotoxic screening test was also performed.

MATERIALS AND METHODS

Animals

Male and female New Zealand White rabbits (2-3 kg) were obtained from Price Rabbitry, New Carlisle, Ohio. Male (200-300 g) and female (150-250 g) Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, Massachusetts. The male and female Hartley derived guinea pigs (300-450 g) were obtained from Murphy Breeding Laboratories, Plainfield, Indiana.

Leghorn hens (Gallus domesticus, Carey-Nick; 300-320 hybrid) 5-7 months of age and weighing between 1.2 and 3.0 kg were purchased from Carey Farms, LaRue, Ohio. The debeaked hens were group housed in 3 by 6' pens to allow for freedom of movement.

Test Materials

Properties of petroleum base stock MIL-H-5606 (MLO 82-585):

Property	Limits
Pour point (max.)	-60°C
Flash point (min.)	82°C
Acid or base No. (max.)	0.10
Color, ASTM Std. (max.)	No. 1

MLO 82-585 has as its base stock naphthenic type petroleum oil and may contain the following additives: viscosity - temperature coefficient improvers, oxidation inhibitors, antiwear agents, and red dye. The specific formula for the finished hydraulic fluid is proprietary.

Properties of the synthetic hydrocarbon base stock, MIL-H-83282 (MLO 82-233):

Property	Limits
Pour Point (max.)	-54°C
Flash Point (min.)	204.4°C
Acid or Base No. (max.)	0.10
Color, Saybolt (min.)	+30
Fire Point	246.1°C

MLO 82-233 has a base stock of a synthetic hydrogenated polyalphaolefin with a nominal $C_{30}H_{62}$ formula. The main additive, approximately 30%, is an ester and may be either a polyolester or a diester. The fluid also contains oxidation inhibitors, antiwear agents, blending fluids, and red dye. The exact composition of the finished fluid is proprietary.

Since both hydraulic fluids contain TCP with an unspecified amount of TOCP, the fluids were analyzed for o-cresol content by the method given in MIL-H-19457C(SH). Following a base hydrolysis of the phosphate esters in a Parr Bomb, the phenolic portion was extracted with diethyl ether. The extract was analyzed

by a gas chromatograph/mass spectrometer method described by MacEwen and Vernot (1982).

The saponified and extracted MLO 82-233 sample showed no o-cresol within the sensitivity of the GC/MS, which was 15 ppm. However, a slight response was noted with the MLO 82-585 sample at the o-cresol retention time of 6.5 minutes (Figure 1). The peak at 7 minutes was identified as m-cresol on the basis of mass spectral data and previous experience with similar hydraulic fluids (MacEwen and Vernot, 1983). The relationship of the two peaks of the total ionization scan compared to that of the 108 atomic mass unit scan indicated that there may be some o-cresol present in the sample of 82-585, but its concentration would be extremely low.

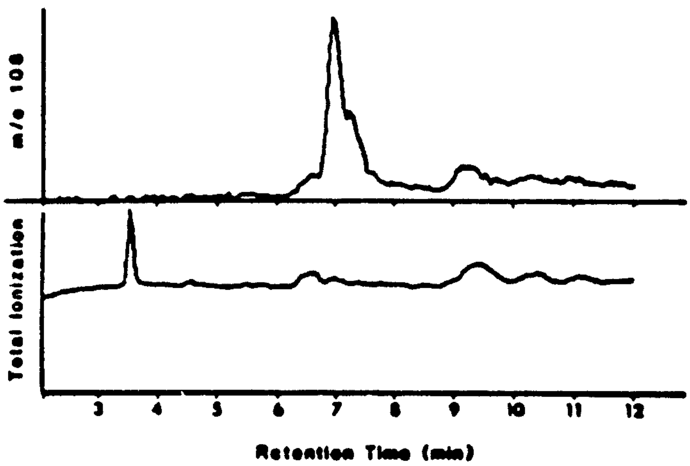

Figure 1. A comparison of total ion and single ion (m/e 108) GC/MS chromatograms of MLO 82-585.

Oral Toxicity

The test materials were diluted with corn oil and administered using glass syringes equipped with ball-tipped oral dosing needles. A dose volume of 1.0 mL/100 g of body weight was used. Test animals were male and female Sprague-Dawley rats. Five rats of each sex were dosed at 5 mL/kg of body weight. The animals were fasted overnight prior to dosing.

Animals were observed frequently on the day of dosing and twice daily during a 14-day posttreatment observation period. Visible signs of toxicity were recorded. Body weights were obtained at the time of dosing and at 1, 2, 4, 7, 10, and 14 days posttreatment.

Dermal Toxicity

Male and female albino New Zealand rabbits were used. The rabbits were clipped as closely as possible with an Oster® clipper equipped with surgical blades and a vacuum attachment.

Five animals of each sex were dosed with 2 mL/kg of body weight. The liquid material was applied undiluted to the back of the rabbit and divided as equally as possible between the two sides. The dose was kept in place by applying 8-ply gauze patches over the liquid on each side of the back. A patch of clear plastic wrap was then applied over the entire clipped back area and elastoplast tape was used to wrap the midsection of the rabbit. Specially designed rabbit restraining harnesses were fitted to each rabbit at the time of dosing and kept in place during the dosing period. These harnesses prevented excessive movement of the rabbits and prevented the rabbit from chewing on the taped area. The harnesses did, however, allow the rabbit access to food and water during the dosing period.

All doses were kept in contact with the rabbit's skin for 24 hours. After 24 hours, the tape, plastic wrap, gauze, and harness were removed. The rabbits were maintained in individual cages postexposure and observed for mortality or other signs of toxicity during the 14 days immediately following exposure.

The animals were observed frequently on the day of dosing and twice daily thereafter. Visible signs of toxicity were recorded. Body weights were obtained at the time of dosing and on days 1, 2, 4, 7, 10, and 14 posttreatment.

Acute Inhalation Toxicity

Concentrated vapors of the test materials were generated in a gas wash bottle equipped with a fritted disk. Dried air was blown at a known rate through the bottle containing a measured amount of test material. The resulting air-vapor mixture was conducted to a 60 liter plastic chamber containing 5 male and 5 female rats. Analytical concentrations were not measured; however, estimated concentrations were calculated by material balance.

The exposures lasted for 6 hours. Animals were observed frequently during the day of exposure and twice daily during the 14-day holding period. Visible signs of toxicity were recorded and body weights were measured on days 0, 1, 4, 7, 10, and 14.

Eye Irritation

One-tenth milliliter of the undiluted test material was applied to 1 eye of each of nine albino rabbits. The opposite eye was untreated and served as a control. The eyes of the animals were examined with fluorescein stain prior to use to ensure absence of lesions or injury. The treated eye of 6 rabbits remained unwashed while the remaining 3 rabbits received test material and then had the treated eye flushed for 1 minute with lukewarm water starting no sooner than 20-30 seconds after instillation. Examination for gross signs of eye irritation were made at 24, 48, and 72 hours postapplication. Scoring of irritative effects was according to the method of Draize (1959) in which corneal, iris, and conjunctival effects are scored separately.

Skin Irritation

A patch test method was utilized to determine the degree of primary skin irritation of intact and abraded skin of albino rabbits.

Six rabbits were clipped of all possible hair on the back and flanks 24 hours prior to exposure to allow for recovery of the skin from any abrasion resulting from the clipping. One of the two test areas on the back was abraded through the stratum corneum, but not sufficiently deep to disturb the dermis or to produce bleeding. This was made in a square pattern with a hypodermic needle used to make the incisions.

Undiluted test materials were applied in the amount of 0.5 mL to the designated patch areas and covered by a 1" square of surgical gauze two single layers thick. The gauze patches were held in place with strips of elastoplast tape. The entire area was covered with polyethylene plastic wrap and secured with more elastoplast tape. These patches remained in place on the rabbits for 24 hours. During that time, the rabbits were fitted with leather restraining collars. After 24 hours, the wrap and patches were carefully removed and the test areas were evaluated for irritation using the Draize (1959) table as a reference standard. Readings were also made at 72 hours (48 hours after the first reading) and at 5 days after application.

Skin Sensitization

A modification of the test described by Maguire (1973) was used. In this test, 10 individually caged albino guinea pigs, 6-8 weeks of age, were used for each material. The materials were tested for primary irritation on 3 guinea pigs by application to the clipped flank. Observations were made at 24 hours for signs of

irritation. If the test material was irritating to the guinea pig skin, dilutions in liquid petrolatum were made to determine the maximum non-irritating concentration.

The test materials were applied at their maximum non-irritating concentration. An area on the back of each animal directly above the forelegs was clipped with electric clippers and chemically depilated with a commercial depilatory ("Neet") on the morning of the first insult exposure. At each application, 0.1 g of the test material was applied to this area on a 1/2 x 1/2 inch cotton gauze square, covered with dental dam, and held in place with adhesive tape. The first insult patch was allowed to remain in place for 2 days, then removed, and a second application of 0.1 g was made. Two days later, this patch was removed, a total of 0.2 mL of 50% Freund's[1] adjuvant in distilled water per animal was injected intradermally, using two or three points adjacent to the insult site, then a new patch of 0.1 g of the test material was applied. On the third day after this application, the patch was removed and a new patch of 0.1 g of the test material applied. The last patch was removed 2 days later, and the animals were allowed to rest for 2 weeks. Each time the insult patches were removed, the condition of the skin at the application site was evaluated and recorded.

After a 2-week rest period, both flanks of the animals were clipped and challenged, one side with the test material and the vehicle (petrolatum) on the other flank. The challenge applications were not occluded. The skin responses at these sites were recorded at 24 hours and 48 hours after application. Any animal showing measurable erythema and/or edema at the test solution challenge site was rated as a positive responder.

Acute Delayed Neurotoxicity Testing

Prior to the neurotoxicity testing, acute oral toxicity tests with chickens were conducted. Each hydraulic fluid was administered to nonfasted hens at a concentration of 5 mL/kg body weight. As no deaths occurred, this concentration was used in the acute neurotoxicity test.

The hydraulic fluids, as well as the positive control TOCP, were administered to unfasted hens in an undiluted state. A negative control group received appropriate doses of corn oil. The method follows the proposed guidelines of the Environmental

[1] Bacto Adjuvant Complete, Freund, Difco Laboratories, Detroit, Michigan.

Protection Agency (EPA, 1982). The following dose regimen was followed:

MLO 82-233:	A group of ten hens received a peroral dose of 5 mL/kg.
MLO 82-585:	A group of ten hens received a peroral dose of 5 mL/kg.
TOCP:	A group of ten hens received a peroral dose of 500 mg/kg.
Corn oil:	A group of ten hens received a peroral dose of 1 mL/kg.

Grading by three observers began on the day following dosing and continued through 21-days after the initial dose.

The following point score was used:

Symptom Free..........................0 Points
Doubtful of Minor Symptoms.............2 Points
Positive Paralytic Symptoms...........8 Points
Advanced Paralytic Symptoms...........12 Points
Death.................................16 Points

During observation and grading, the hens were removed from their enclosure and placed on a rubber mat to provide sure footing. Symptoms of test hens noted during the observation period were compared with those seen in the TOCP treated hens. All hens were weighed prior to dosing and once weekly thereafter.

All test and control hens were examined for gross pathology at death. Longitudinal and cross sections of the spinal cord (cervical, lumbar, and thoracic regions) and a section of the sciatic nerve were sampled for histopathology examination.

RESULTS

Acute Oral, Dermal, and Inhalation

Neither hydraulic fluid produced mortality by the oral, dermal, or inhalation route at the maximum concentration tested as shown in Tables 1 and 2. Toxic signs were absent and mean body weight gains were normal during the subsequent 14-day observation period.

TABLE 1. ACUTE ORAL, DERMAL, AND INHALATION TOXICITY OF MLO 82-585

Route of Exposure	Species	Sex	Dose	No. Dead/No. Dosed
Oral	Rat	M	5.0 mL/kg	0/5
Oral	Rat	F	5.0 mL/kg	0/5
Dermal	Rabbit	M	2.0 mL/kg	0/5
Dermal	Rabbit	F	2.0 mL/kg	0/5
Inhalation	Rat	M	1148 mg/m^3 [a]	0/5
Inhalation	Rat	F	1148 mg/m^3 [a]	0/5

[a] Concentration calculated by dividing the amount of test material used by total air volume.

TABLE 2. ACUTE ORAL, DERMAL, AND INHALATION TOXICITY OF MLO 82-233

Route of Exposure	Species	Sex	Dose	No. Dead/No. Dosed
Oral	Rat	M	5.0 mL/kg	0/5
Oral	Rat	F	5.0 mL/kg	0/5
Dermal	Rabbit	M	2.0 mL/kg	0/5
Dermal	Rabbit	F	2.0 mL/kg	0/5
Inhalation	Rat	M	1130 mg/m^3 [a]	0/5
Inhalation	Rat	F	1130 mg/m^3 [a]	0/5

[a] Concentration calculated by dividing the amount of test material used by total air volume.

Eye Irritation

Neither hydraulic fluid caused ocular irritation in the rabbits. No differences were noted when comparing the exposed eyes, washed or unwashed, with the respective control eyes at the scheduled observation periods.

Skin Irritation

The hydraulic fluid MLO 82-233, when applied undiluted to intact and abraded skin, did not produce a primary irritation response.

The hydraulic fluid MLO 82- 5 was found to be a moderate, reversible primary skin irritant (Table 3). The 72-hour primary irritation index was calculated to be 2.1 which would classify this fluid as a moderate skin irritant. Five days following treatment, no erythema or edema was present. There was, however, minor eschar noted in all 6 rabbits.

TABLE 3. PRIMARY SKIN IRRITATION OF MLO 82-585

Mean Skin Reaction Scores

Time Post Dose (Hr)	Erythema[a] Intact	Erythema[a] Abraded	Edema[a] Intact	Edema[a] Abraded	Necrosis[b] Intact	Necrosis[b] Abraded
24	1.2	1.5	0.5	1.7	0	0
72	1.7	1.7	0	0.2	c	c
Day 5	0	0	0	0	c	c

Primary irritation index 2.1[d]

[a] Erythema and edema - scored on a scale of 0-4 with 4 being the most severe.
[b] Necrosis - scored 0, 5, 10, 15 with 15 as the largest area of necrosis.
[c] Minor eschar formation.
[d] No. of animals x Total reaction score (erythema and edema).
(No. of animals x No. of observations x No. of test sites).

Skin Sensitization

In the preliminary screening tests, neat MLO 82-233 and a 1% solution of MLO 82-585 in liquid petrolatum were found to be non-irritating. The results of the sensitization testing are presented in Table 4. These data indicate that neither of the hydraulic fluids is a potential skin sensitizer.

TABLE 4. SKIN SENSITIZATION POTENTIAL OF MLO 83-233 AND MLO 82-585

Test Material	Conc. %	% Demonstrating Positive Response Post Challenge			
		Control Side		Treatment Side	
		24 hr	48 hr	24 hr	48 hr
MLO 82-585	1.0	0	0	0	0
MLO 82-233	100	0	0	0	0

Acute Delayed Neurotoxicity

Mean body weights of the test hens were not significantly different ($p < 0.05$) from those in the corn oil control group. Positive neurotoxic symptoms were observed in all hens receiving TOCP. A TOCP dosed hen was sacrificed after 17 days due to paralysis and inability to obtain food and water. All hens in the corn oil control group exhibited negative or doubtful symptoms, as did the hens receiving MLO 82-585.

Nine of the 10 hens reciving MLO 82-233 showed negative or doubtful signs through the 21-day period. One hen appeared normal through 19 days; however, when observed on day 20 it refused to walk and appeared paralyzed. On the following day the hen was able to stand but showed definite signs of leg weakness. It was not possible to determine if the partial paralysis was accidental or chemically induced. X-rays failed to demonstrate significant musculoskeletal changes, and gross pathology was essentially normal.

Because of an equivocal response at 5 mL/kg, the MLO 82-233 neurotoxicity test was repeated increasing the dose level to 7.5 mL/kg and extending the observation period through 30 days, excluding weekends.

There were no significant differences ($p < 0.05$) in mean body weights between the groups at any weighing period. However, a slight decline in mean body weight of the TOCP positive control group of hens was seen at the 14-day weighing period which corresponded with the appearance of neurotoxic signs. The low mean body weight of TOCP hens continued through the remainder of the 30-day observation period. The MLO 82-233 hens showed a slight decline in mean body weight at the 21-day weighing but recovered by 28 days.

Neurotoxic symptoms were observed in all hens that received TOCP. Two TOCP dosed hens were sacrificed after 24 days due to

paralysis and inability to obtain food and water. The corn oil control group resulted in all negative or doubtful scores.

Nine of the 10 hens receiving MLO 82-233 showed negative or doubtful signs through the 30-day period. One hen appeared normal through 11 days; however, on day 12 (not a scoring day) the animal technician noted that the hen displayed poor coordination and loss of balance. By day 13 the hen was prostrate and was killed on day 15 due to its moribund condition.

Histopathologic examination of nerve tissue revealed neuronal degeneration occurring sporadically in hens exposed to both hydraulic fluids. Three hens (30%) intubated with MLO 82-585 and 1 hen (10%) intubated with MLO 82-233 exhibited degenerative changes. These changes consisted of (1) mild segmental axonal swelling and ballooning of the associated myelin sheath (B2 or B3 in tables) and (2) dissolution of the Nissl substance (chromatolysis) of cell bodies (ND in tables). The neuronal degenerative changes were similar to the lesions produced by the positive (TOCP) control; however, the distribution and severity of degeneration in TOCP-exposed hens was significantly greater. In addition, negative (corn oil) control hens also exhibited similar changes (30% in both studies). The lesions noted in nerve tissue sections from all treatment groups are listed for comparison in Tables 5 through 11. Minimal lesions (designated as 1 in the tables) were reserved for changes in the myelin sheath only, whereas the range of mild to moderate severity codes (2 and 3) represent increasing damage to the axon as well as the surrounding myelin. Degenerative changes in the neuronal cell body (ND) are also annotated in the tables. The cross section annotation is used primarily to designate the location of the lesions in the spinal cord.

Exclusive of specific axonal lesions, a minimal, segmental disruption of the myelin sheath of nerve fibers was observed in a high percentage of control and exposed animals. This change was characterized by a slight ballooning of the myelin sheath which usually contained a clump of degenerating myelin. The change was always multifocal in distribution and minimal in severity (coded B-1 in tables) and occurred sporadically throughout the cord with no specific predilection for any specific region. Special staining (Kluver-Barrera) demonstrated the change to be confined to the myelin sheath with little apparent effect on the axon. The precise significance of this change could not be ascertained and normal turnover of myelin and/or artifact can not be discounted as a possible causative factor.

TABLE 5. GRADED LESIONS[a] IN NEURAL TISSUE AFTER CORN OIL SINGLE-DOSE ADMINISTRATION; 21-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
3356	NL	B1	NL	B1	NL	B1	NL
3357	LF	B1	NL	B1	NL	B1	NL
3310	NL	NL	NP	NL ND	NL	B1	B1
3311	NL	B2[d]	NP	B1	NL	NL	NL
3312	NP	NL	NL	B1	NL	B1	NL
3319	NL	B1	NL	B1	NL	B1	NL
3320	NL	B1	NL	B2	NL	B1	NL
3321	NL	NL	NL	B1	NL	NL	NL
3354	NL	B1	NL	B1	NL	B1	NL
3355	NL	NL	NL	NL	NL	NL	NL

[a] ND - Neuronal Degeneration; NL - No Lesion; NP - Not Present; LF - Lateral Funiculus; FA - Focal; B - Multifocal; 1 - Axonal Degeneration, Myelin Only; 2 - Axonal Degeneration, mild.
[b] Cross section.
[c] Longitudinal section.
[d] Degeneration, axon w/astrocytosis.

TABLE 6. GRADED LESIONS[a] IN NEURAL TISSUE AFTER TOCP SINGLE-DOSE ADMINISTRATION; 21-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
3352	LF	B2	NL	B2	ND	B2	B1
3353	LF	B2	NL[d]	B2	ND	B1	NL
3298	LF	B3	NL	B2	NL	B1	B2
3307	LF	B2	NL	B2	NL	NL	NL
3308	LF	B2	LF	B2	ND	B2	B2
3309	LF	B3	LF	B2	VF	B2	NL
3322	NP	B2	NP	B2	NP	ND	B1
3323	LF	B2	LF	B2	VF ND	B2	B2
3324	NP	B1	VF	B2	NL	NL	NL
3351	LF	B3	L&VF	B2	NL	ND	NL

[a] ND - Neuronal Degeneration; NL - No Lesion; NP - Not Present; LF - Lateral Funiculus; VF - Ventral Funiculus; B - Multifocal; 1 - Axonal Degeneration, Myelin Only; 2 - Axonal Degeneration, mild; 3 - Axonal Degeneration, moderate.
[b] Cross section.
[c] Longitudinal section.
[d] Astrocytic foci B1.

TABLE 7. GRADED LESIONS[a] IN NEURAL TISSUE AFTER MLO 82-585 SINGLE-DOSE ADMINISTRATION; 5 ML/KG, 21-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
3349	NL	NL	NL	B1	NL	NL	NL
3350	NL	B2	NL	B1	NL	B1	NL
3304	NL	NL	NL	B1	NL	NL	NL
3305	NL	B1	NL	B1	NL	B1	NL
3306	NL	NL	NL	B1	NL ND	B1	NL
3325	NL	B1	NL	NL	NL	ND	NL
3326	NP	NL	NL	B1	NL	B1	NL
3327	NL	B1	NL	NL	NL	B1	NL
3328	NL	B1	NL	B1	NL	B1	NL
3348	NL	B1	NL	B1	NL	B1	NL

[a] ND - Neuronal Degeneration; NL - No Lesion; NP - Not Present;
B - Multifocal; 1 - Axonal Degeneration, Myelin Only;
2 - Axonal Degeneration, mild.
[b] Cross section.
[c] Longitudinal section.

TABLE 8. GRADED LESIONS[a] IN NEURAL TISSUE AFTER MLO 82-233 SINGLE-DOSE ADMINISTRATION; 5 ML/KG, 21-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
3346	NL	B1	NL	NL	NL	B1	NL
3347	NL	B1	NL	B1	NL	B1	NL
3300	NP	NL	NL	B1	NL	B1	NP
3301	NL	B1	NL	B1	NL	B1	NL
3302	NL	B1	NL	B1	NL	B2	NL
3303	NL	B1	NL	B1	NL	B1	NL
3329	NP	B1	NL	B1	NL	B1	B1
3330	NL	B1	NL	B1	NL	NL	NL
3331	NL	B1	NL	B1	NL	B1	NL
3345	NL	B1	NL	B1	NL	B1	NL

[a] ND - Neuronal Degeneration; NL - No Lesion; NP - Not Present;
B - Multifocal; 1 - Axonal Degeneration, Myelin Only;
2 - Axonal Degeneration, mild.
[b] Cross section.
[c] Longitudinal section.

TABLE 9. GRADED LESIONS[a] IN NEURAL TISSUE AFTER CORN OIL SINGLE-DOSE ADMINISTRATION; 30-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
4589	NL	NL	NL	B1	NL	B1	NL
4590	NL	NL	NL	B1	NL	B1	NL
4591	NL	B1	NL	B1	NL	ML	NL
4592	NP	A2	NL	B1	NL	B1	NL
4593	NL	NL	NL[d]	B2	NL	NL	NL
4594	NL	NL	NL	NL	NL	NL	NL
4595	NL	B1	NL	B1	NL	B1	NL
4596	NL[e]	NL	NL[e]	B1[e]	NL	B1	B2[f]
4597	NL	NL	NL	B1	NL	NL	NL
4598	NL	NL	NL	NL	NL	NL	NL

[a] NL - No Lesion; NP - Not Present; A - Focal; B - Multifocal; 1 - Axonal Degeneration, Myelin Only; 2 - Axonal Degeneration, mild; 3 - Axonal Degeneration, moderate.
[b] Cross section.
[c] Longitudinal section.
[d] Proliferation, astrocytic, B1.
[e] Perivascular cuffing, B2.
[f] Lymphoid nodules, B3 perivascular and perineural.

TABLE 10. GRADED LESIONS[a] IN NEURAL TISSUE AFTER TOCP SINGLE-DOSE ADMINISTRATION; 30-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
4587	NL	NL	NP	B2	ND ND	B2	NL
4588	LF	B2	NL	B1	ND	B2	NL
4584	NL	B2	D&LF	B2	NL	B2	NL
4585	LF	B2	NL	B2	NL	B1	NL
4583	NL	B2	NP	VF	A2	NL	
4581	NP	B2	NL	B2	NL	B3	A2
4580	NL	B2	NL	B2	NL	NL	NL
4579	NL	B2	NL	B2	NL	B2	NL
4527	LF	B2	VF	B2	NL ND	B2	B2
4526	LF	B3	LF	B2	NL	NL	NL
4437	NP	NL	NL	B1	NL	NL	NP

[a] ND - Neuronal Degeneration; NL - No Lesion; NP - Not Present; DF - Dorsal Funiculus; LF - Lateral Funiculus; VF - Ventral Funiculus; A - Focal; B - Multifocal; 1 - Axonal Degeneration, Myelin Only; 2 - Axonal Degeneration, mild; 3 - Axonal Degeneration, moderate.
[b] Cross section.
[c] Longitudinal section.

TABLE 11. GRADED LESIONS[a] IN NEURAL TISSUE AFTER MLO 82-233 SINGLE-DOSE ADMINISTRATION; 7.5 ML/KG, 30-DAY OBSERVATION

Hen	Cervical		Thoracic		Lumbar		
#	Cx[b]	Lx[c]	Cx	Lx	Cx	Lx	Sciatic
4599	NP	NL	NL	B1	NL	B1	NL
4600	NL	B1	NL[d]	NL	NL	NL	NL
4601	NL	NL	NL	B1	NL	B1	NL
4602	NL	NL	NL	NL	NL	NL	NL
4603	NL	NL	NL	NL	NL	NL	NL
4604	NL	B1	NL	B1	NL	NL	NL
4605	NL	NL	NL	NL	NL	NL	NL
4606	NL	NL	NL	NL	NL	NL	NL
4607	NP	NL	NL	NL	NL	B1	NL

[a] NL - No Lesion; NP - Not Present; B - Multifocal; 1 - Axonal Degeneration, Myelin Only.
[b] Cross section.
[c] Longitudianl section.
[d] Inflammation, lymphocytic, perivascular, B1.

One animal (3302) exposed to 5 mL/kg MLO 82-233 exhibited both clinical and histopathologic signs of neuronal degeneration. However, the neuronal damage observed histopathologically was relatively insignificant in comparison to TOCP-exposed animals. Other possible causes of neuronal degeneration must be considered in this animal, although none were identified in the sections examined. Furthermore, no histopathologic evidence of neuronal damage was noted in the second group of animals exposed to the greater dose of MLO 82-233. The changes observed in MLO-exposed animals were comparable to those seen in the negative control groups and were relatively insignificant when compared with the changes in positive control groups.

CONCLUSIONS

MLO 82-233 and MLO 82-585 caused no mortality when administered orally or dermally. Neither material caused mortality among male and female rats exposed to saturated vapor concentrations for single 6-hour periods and neither material was found to be an eye irritant. MLO 82-585 was determined to be a moderate skin irritant, while neither hydraulic fluid demonstrated skin sensitization potential.

Although 2 hens displayed signs of leg weakness and incoordination, histopathologically, the changes noted were not the classical changes of delayed neuropathy as seen in the hens given TOCP. The results of the acute toxicity tests reported herein indicate that neither hydraulic fluid should pose any hazard to workers during production or use.

REFERENCES

Beresford, W. A. and P. Glees (1963), Degeneration in the long tracts of he cords of the chicken and cat after triorthocresylphosphate poisoning, Acta Neuropathol., 3:108-18.

Doull, J. D., C. D. Klassen, and M. D. Amdur, eds. (1979), Casarett and Doull's Toxicology: The Basic Science of Poisons, MacMillan Publishing Company, New York.

Draize, J. H. (1959), Dermal toxicity, Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, The Staff of the Division of Pharmacology of the Federal Food and Drug Administration, Austin, Texas, The Editorial Committee of the Associates of Food and Drug Officials of the United States, p. 51.

EPA (1982), Environmental Protection Agency, Health Effects Test Guidelines, Report 560/6-82-001.

MacEwen, J. D. and E. H. Vernot (1982), Toxic Hazards Research Unit Annual Technical Report, AFAMRL-TR-82-62, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio (AD A121717).

MacEwen, J. D. and E. H. Vernot (1983), Toxic Hazards Research Unit Annual Technical Report, AFAMRL-TR-83-64, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio (AD A136170)

Maguire, H. C. (1973), The bioassay of contact allergens in the guinea pig, J. Soc. Cosmet. Chem., 24:151-162.

* U.S. GOVERNMENT PRINTING OFFICE: 1985 - 646-006/20231